April 30, 1953

Dear Bruce:

Your letter of April 9 and the two halves of the ms. all arrived during the past week, the firstnamed marked "insufficient postage for air-mail", which accounts for what may seem like an unconscionable delay in my reply. Norton visited a couple of weeks ago, and we are in substantial agreement in our views on the ms., in particular that while we do not wish to delay you any further, the ms. can still stand considerable condensation. My own view is that you have met every major issue I may have raised (barring any further strong comment below), and will now leave further questions that may be raised to your own judgment. If the editors of JGM are willing to accept the paper as is, it may well be left at that; if not (as I would strongly suspect) you may have a job of surgery still on your hands. At any rate, there is every reason to get this in the press. We should look ahead to the problem of reprints, as this may have to be decided rather suddenly when proof comes. This may seem exaggerated, but I think we have to anticipate a demand on this side of the Atlantic of at least 750 reprints. Of these, Norton should have 200; I expect most of the stateside requests will be directed here, and it would be rather silly to forward them to London, but if you would prefer to handle all responses to request cards, you could cut my figure from 750 to about 550. My general mailing list takes about 350-450. Before making a definite commitment, I had better have at least a rough estimate of the costs. If you can get this, plus your own estimate of how many you expect to get yourself it will help on this recurrently vexatious business. (I might add that Norton and I shared 650 reprints of the Z&L, and the supply has long since been exhausted, except for a contingency reserve (for future students, etc.))

To take up your letter first (let me parenthesize that I don't hope to get an overall view of a letter like this, and must follow your own point by point. Anything not mentioned has been noted and is, presumably ok.

";" Don't pay much attention to the ones I remarked: they are essentially a random sample. The address, acknowledgments seem ok. Treatment of transduction vs. K-12 story is ok, as is. Transduction is defined at p. 681 (Z&L) as "genetically unilateral transfer in contrast to union of equivalent elements in fertilization. The working hypothesis that FA is an agent of genetic transduction provides....", and in Physiol. Rev. 32:413 as "restricted transfer of genetic material to the cell". If you want to see why I emphasize this terminology, see Dobzhansky's comment on E-Taylor as "progress on the road towards the induction of specific mutations in specific genes" (Amer. Natur. 87:123) which propagates the error in his monograph. I fully accord with "pneumococcus transformation" as a designation for that particular case, for historical reasons, and will have no complaint if you do not explicitly subsume this under transduction, so long as PnT is not contrasted with Sal. Transd. I pose the hypothetical question: what happens if we succeed in extracting the genetic fragments from the phage particles, and can inject them by some other means? The concept of transduction as the overall mechanism is the only one sufficiently general to cover the whole situation. I regret my error in calling the transfer of F state as a transduction. Perhaps I can wiggle out of it by setting aside "genetic transduction" as the distinctive term, and leaving transduction to its dictionary meaning. There is ~~obviously~~ no common ground between the F transfer and fertilization (from a genetic point of view) that requires a contrasting terminology.

I was delighted to hearaabout the Glasgow strains. If anything, the agreement between genotypy and lysotypy should be emphasized even further, as bolstering both. How about specifying NTC 3047 for Glasgow O.

mportant) ** In view of the current hassle over Salmonella nomenclature, I think it would be most hazardous to describe species (cf. Joan Taylor and the British Enter. Subcomm. in the Int. Bull. Bact. Nomen....) Why not just serotype, type, or serological type?

"gene" is taken too seriously by some; "genetic factor" is less insistent as an absolute unit, and to my ears just sounds better in the absence of a complete discussion of the "gene theory".

"combinatorial" still sounds adequate. You have tried every combination of bacterium with FA: there is no question of permutation with non-equivalents, to be fussy. I.E. a x- b is the same as b x- a. Dictionary usage also gives, e.g. "combinatorial analysis= math. study of permutations and combinations."

I don't dislike your suggestion about Fla_1--H_1. I am suspicious of it only because it is too obvious. To talk about predictions, would would think that H_1--H_2 would be even more likely linked, but there is no sign of this. The present version is sufficiently cagey. Would you prefer to quote the more general discussions of pseudo- and para-alleles (References: ~~later, when I get back to lab~~ Taku-Komai, Amer, Nat. 84:381; Laughnan, more recently in the same; several papers in CSH 51: Bonner, Lewis, Stephens, Giles, ~~Pontee~~-- the first is probably sufficient). Frankly I am not yet entirely convinced that this is more than a coincidence, even considering SW-553

I have a record of motilizing: SW970--x SW545, and SW972--xSW541. We should develop other markers in all the standard testers. May I suggest you do this for [redacted] TM's , and I'll keep the others in mind. I've gotten only j phases from pullorum, gallinarum --x H901. This doesn't mean much. Altogether, using 543Fla+ --x SL13, I've gotten just one or two swarms (both a), nothing with Fla_1- --x SL13. However, in the course of some track isolations, I picked up some derivs. of SL13 which may show a higher frequency of transduction. If so will send you these (and repeat linkage tests). These experiments were designed to see whether tracks were transductions initially abortive, or crossovers with a residue, e.g. distinguishing SW666 and SW553 as Fla_1 and Fla_{1a}, tracks were picked from 1- H_1^b --x 1a- H_1^{gp} to verify whether all the tracks were still 1a- H_1^{gp}, or some possibly 1- or 1a- H_1^b (temporarily motile by a residual crossover fragment). In quite a large experiment, involving all the feasible combinations, no such crossovers were found. However, some of the tracks from TM2--x SL13 seem to be more amenable (possibly simple selection for better transinducible cells), to subseuqent transduction when they were tested. The experiment was motivated by Morse' result, who has found that some of the unstable Gal+ from Gal_1- --x Gal_4- split off occasional Gal_1^0 as well as Gal_4-.

your p. 4 con'd: Yes. TM2 --x SW971 gave gm. (Culture unrelated to 970, 972). 970 and 972 may possibly be the same, am tracing history. 972 come from Kauffmann from Floyd from fresh eggs in Cairo. Both are gm.

I would prefer TM2, just as a strain label. Norton agrees. I would indeed like to hear details about origin of O901-- can't find it in print, and have been meaning to ask Felix. (Met Anderson at Urbana last week: he didn't know wither. Anderson will be here in about two weeks. I might interpolate that we bought a tape recorder from him which he picked up in NY, and later found could not use on train electric circuits).

Wunderbar! on track cell.

We have abandoned hope of going to Europe this summer. Have no preference or objection to whatever you might like to present, joint or separate (if former is based on this paper). I am going to ask Cavalli since he has sought my advice) to invite you to give a longer paper at Rome. As to MGB, however, I dissent (but will not insist). I just don't see any point to a preview which is going to come out in full detail. Please don't quote my own past sins, but I have become rather sour about MGB, which is now neither fish nor foul (private circular vs. publication).

I agree about leaving out SW553, 970, 972. You have to stop somewhwre.'

SW535 = S. stanley, Edwards #15. I think you or I had done TM2 --x SW535 at Madison, with the same result.

Before this letter is buried in ms. details, may I ask whether you ever streaked out the SW684 (unstable Gal+ transduction) which I believe I did send you some time ago. Our own culture seems to have gone to pot (mixture of pure + and -, no Galv), and I would appreciate it getting it back, if you have it. Also, as mentioned further, I have to give up TM2 for phase variation studies. Have started with SL46 as the most stable in your 1949 series with approx = back and forward, but will eventually want to compare different strains. Could you send me a batch of those for whichyou had measur rates? Finally, have you ever looked at the S. enteritidis NTC 3045, mentioned by Schütz I would appreciate the strain and its history, if available. Are there any more O forms floating about in NTC? The Army evidently threw out a bunchbthat Bruner collected durin the war. Also, LeMinor recently published one in Ann. Inst. Pasteur (typhi)-- have you got hold of it? Which reminds me, did you ever perfect a technique for distinguish O and H on agar, without excessive overgrowth? We don't seem to be able to hit the right agar concentration (plate to plate variance very high), and methocel did not work.

I am beginning to believe that TM2 goes through 3 distinct phases: i, i+1,2, 1,2. The last is rather unusual. Also, the i+1,2 phase seems to be distinctly more motile than the i. Edwards quotes it as a fairly common occurrence that one phase is much less motile than the other, and I think I can confirm this for several cases, especially with artificial phases like z_{22}. The phases may yet have distinct adaptive values not directly concerned with their antigenicity. Let me add that abortus-equi --x TM2 has given an i+enx from which I have been unable to isolate anything else. The i agglutination is usually delayed (even with cultures passed through enx serum), but both the i and enx agglutinations seem complete. I have some microscopic studies under way to check on this phase confusion.

------- Now the paper,

I note your difficulty with species vs. serotype. I see no reason not to use the binomials, but to refer to them as serotypes (without making any point of it).

There are two things, generally, which dilute the paper (aside from a prolixity of style which is entirely a matter of taste). First is the adoption of a duplicate terminc logy, one bacteriological, one genetic, with the terms repeatedly apposed. I think the latter can be dropped, or once defined used to the exclusion of the former. Second is a repetition of general statements about the transduction of individual factors, the divorcement of phage from FA, the trails as abortive transductions, inter alii. There are often good rhetorical reasons for such repitition, but the writing here reaches such length that redundancies should be excised. These may be mentioned particularly below. Some of the experiments are given in excessive detail, e.g. the method of preparing phage. But as indicated before, these are items most of which can be correct (as the editors may well insist) on the advice of the referee.

Specific items are cited by page and cm. from top of page.

4:20 customary for easy. 5:1 serotypes/~~spice~~ species.....

5:19 "extent of flagellation"— what has been measured is usually H-agglutinability, or motility. Can you document these as mutative? (~~except~~ the slow spreader ty is explicitly given as having normal flagellation). Do you have in mind your masked H?

** 6:11 my own findings support phase variation as a sort of reversible differentiatic I may be embarrassed later at this phrase, although it is compatible with the loosest denotation of "mutation". Would you be willing to delete "the process consists of mutation and reverse-mutation", ~~and sub~~ which does not tell your bacteriological reade very much, and substitute variation/mutation in statement about rate? Alternatively, you might have to qualify your meaning of mutation, which would be awkward for what worth.

6:bottom TM2/L~~2~~... 7:23 a minor example of redundancy:"excessive dose phenomonen" is superfluous, reference sufficient.

~~flagellated~~

~~10~~ 8:10 this phage attacks many Salmonella strains, regardless of their serotype, but only when they have flagella.

10:16 ~~I agree roughness does not~~ "subculture" is not quite clear enough. Emphasize numerous single colony isolations. Ex: "~~Contrary to expectation~~- Extensive single colony isolations from flares crowded with microcolonies always gave stable, motile subcultures similar to those obtained...."

10:23 I agree that roughness does not explain flares. However, since flares are found when rough motile bacteria are inoculated, the flares have no definite connection with transduction, and therefore do not need to be elaborated on here.

26: has/have

~~these of~~-

15:17 typical of/the species (or serotype) is perfectly correct, and less clumsy.

16:32 and elsewhere. How about : for diphasic variation (better than ; which I see is in your table).

18:10 flagellation provides a valid method, which may be of practical value, for ~~determination of species~~ typing stable O strains....
Does not have to be keyed to differences in pathogenicity, which are not entirely reliable anyhow. If typing is of practical value, so is this and no special justif. required.

18:26 etff. How about the subjunctive: lacked etc.

19:30 Lederberg et al 1951 or much better Lederberg, Genetics in the 20th Century, for the several Lac loci. Not L&L.

20:20 I'd rather not express a judgment of propriety. Three strains were determined to have the Vi B (V in the Kauffmann-White scheme) antigen.

gain

20: I find it more a strain to postulate the double coincidence of ~~loss~~ of V and ~~recurrence~~ infection with A2 than the recurrence of Fla-. Your next to last sentence is fine. I would delete the last. (Can you document the variation in V, independent of IV XII? It should be demonstrated experimentally in this strain to support the hypothesis.

23:7 the same/a single~~23:7~~/ 14 not

25:27-8 suggest that strain Glasgow could.... (past tense made me think you were citing Schutze, at first reading)~~28~~

28:12 a vaccine is usually understood (in US) as a modified "virus" used to elicit protective antibody. how about "as agglutinogens (or antigens) for the production of diagnostic reagents."-- this includes contingency of use as as a diagnostic antigen as well.

30: I thought SW-553 was out. 29--31 rep.

31: 13 occurred only exceptionally 32:P2 document.

33: the concept of fragment-transduction should be made ~~exep-pl~~ explicit at the very beginning; otherwise reader gets to think of transfer of"single genes" and the linkage gets to be a shock. Norton and I had some disagreement about this, but I have never believed in the reality of "single genes"as physical units, least of all in transduction. See Z&L 695 (Also see New Yorker, 29(10):102, 4.25.53).

33-bottom. Lac_4 /Lac_2.

33 et ff. linearity is overemphasized. For the first-ebid evidence of it we should have to show that in a 3-factor group, only one of three possible arrangements is consistent with the results. There is some hope of this in SW553--X SW666, but it is not very strong. The geometry of the genotype does not have to be specified now. You have said all there is to say when "in all organisms ...the genes behave as if arranged in linear groups, which correspond to chromosomes (in every thoroughly studied organism)", and a similar organization may be predicated for Salmonella.

2 34 Divorcement. (Cf. p. 11). It was also noted in --x S. typhi but not mentioned by Z&L
Trails Cf. 12
Double transduction Cf. 26.
Why not summarize in one line, and refer back, as major bearing on transduction per se.

35:12 "gene structure" is awkward(do you mean internal structure?)-- genetic structure, or more explicitly, chromosome

:16 "physico-chemical" is pretty fancy. Why not "The vector of transduction in Salmonella is (evidently) a phage particle", and not mislead anyone into thinkin the phage is the active agent, rather than passive carrier.

36:P1 Actually Griffith claimed that ~~xxxxxxxxxxxxxxxxxxxxxxxxx~~ S_y--x R_x

gave S_x as well as S_y. This does not seem to have been followed up.

Actually, not a great many different R strains have been used in Pn. and these have always been selected for absolute stability, which may have something to do with it. (Norton said something that suggests Hotchkiss may be running into this again).
P2 E-Taylor's suggestion of a linear arrangement does not bear discussion. It does not strengthen a case to quote insubstantial evidence. Dele "The data suggested...." I would also dele the next sentence unless you want to discuss this casuistry.

37:7 the genetic fragments /genes. 37:11 National Institutes. Dele "Agr. Exp. Sta.

That's all for now. Forgive the ferocity, which is only a conditioned reflex to mss. these days.

Sincerely,

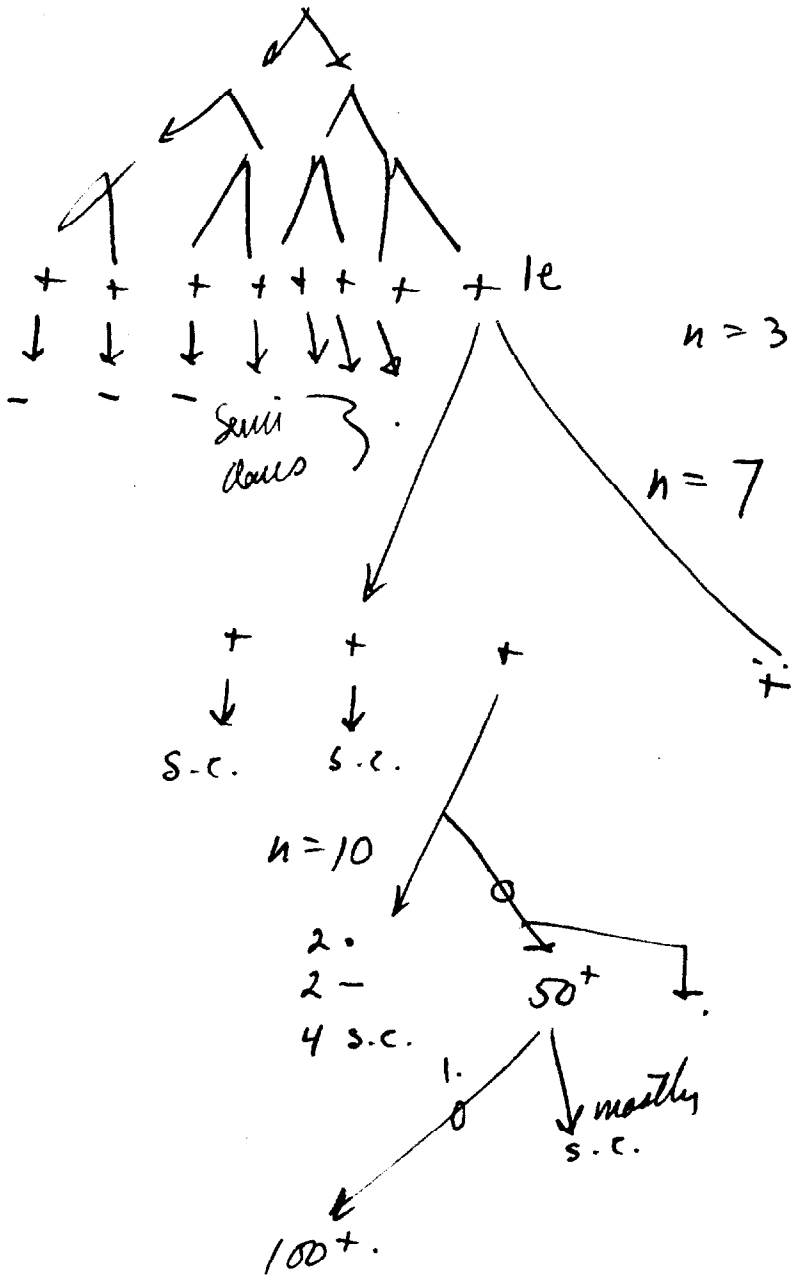
Joshua.Lederberg

P.S. Dr. Dixie Lee Ray (U. of Wash.) who spent a few weeks at Madison has been doing some interesting things on the agglutination of motile and paralyzed, but not of non motile TM by an amoeba (Hartmanella). She'll be in London latter part of July and I have suggested she look you up.

Have you seen Spicer lately? He's been quite ill.

Do you happen to have access to a dictating machine (Edison Voicewriter?) If so we could excahnge disks, which would be more fun. Or, if necessary but more awkward, magnetic tapes.

4/53



n at at least 20th. question.

$n > 15$ (how large?)
-20

$n = 15 + 6$ minimum.